PE1.65 The efficiency of Vitamin D3 supplementation to counteract the negative effects of beta agonists on meat quality of feedlot cattle 409.00

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Abstract—Beta agonists are known affect the ageing potential of beef muscle negatively. On the other hand controlled electrical stimulation of carcasses as well as supplementation of ultra high levels of vitamin D3 for short periods before slaughter could enhance the ageing potential of beef. In this study various levels and durations of Vitamin D3 supplementation in combination with electrical stimulation (ES) after killing were to overcome tenderness problems of beta agonist treated animals were evaluated. Ten out of sixty young steers received no beta agonist (C) while the rest in groups of ten received a beta agonist only (Z) or the last 30 days on feed) or a beta agonist as well as 7x 106 IU/animal /day vitamin D3 for three days (3D7M, six days, (6D7M), six days followed by 7 days withdrawal (6D7M7N) or 1 x 106 IU/animal /day vitamin D3 for nine days (9D1M). Carcass sides (one of each animal) were either electrically stimulated after dressing and splitting of the carcass or left unstimulated. Warner Bratzler shear force and miofibrilar lengths were measured on loin samples aged for three and 14 days. Beta agonist treated loins were significantly tougher than control loins irrespective of ageing period. Vitamin D3 did not improve the tenderness of beta agonist treated loins. Both ageing and ES improved meat tenderness significantly and showed a slightly larger benefit for Z and vitamin D3 treatments than for C. Vitamin D3 do not seem to overcome tenderness problems experienced with beta agonists and more success will be experienced with ES and prolonged ageing.

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Index Terms— Electrical stimulation, tenderness, vitamin D3, Zilpaterol.

I. INTRODUCTION

A large proportion of South African feedlot cattle are supplemented with a beta agonist to improve feed efficiency and yield. Beta agonists are known to affect meat tenderness (and other quality traits) negatively due to an increase in calpastatin activity [11]. Electrical stimulation (ES) could improve meat tenderness by increased activation of the calpain system [8]. In addition, electrical stimulation and post mortem aging combined had a more pronounced effect on the improvement of tenderness of loins from beta agonist supplemented animals than loin from control animals [19]. Under commercial conditions, beef quality is compromised if correct slaughter and post slaughter practices are not in place. A large portion of the cattle industry is integrated so that feedlot owners also run their own abattoirs and processing plants and are able to control much of the processes involved in the final quality of meat. For this reason other methods have recently been implemented by feeders to overcome the potential negative effect of beta agonists in particular and also to improve meat quality in general. One of these practices is the supplementation of very high levels of vitamin D3 a few days prior to slaughter. Various researchers have found that supplementing extremely high levels of dietary vitamin D3 for a limited time prior to slaughter improved meat tenderness by increasing blood calcium levels which play an important role in activating the calpain protease system ([9], [10], [13], [14]). However, reports are not consistent with regards to the level and duration of supplementation as well as the magnitude of the effects. In addition, no studies have reported on ultra-high levels of vitamin D3 to overcome the negative effects of beta agonists. It is also yet to be verified if the effect of electrical stimulation and vitamin D3 are additive.

II. MATERIALS AND METHODS

Sixty young Bonsmara steers (~9 months) were purchased, processed and raised in the research feedlot facilities of the Agricultural Research

Council (Irene, Gauteng Province) on a commercial feedlot diet (120 days). The animals were identified and allocated to six treatment groups of 10 animals each so that the average weight and variation for each group was the same. The control (C) receive no beta agonist or vitamin D3, while the five remaining groups (n=50) were supplemented with the beta agonist, zilpaterol hydrochloride, (Intervet/Schering-Plough Animal Health, South Africa) at 0.15 mg/kg live weight for thirty days during the final weeks of finishing. One group only received zilpaterol (Z), while the other four groups received zilpaterol and vitamin D3 at the following levels and durations before slaugher: 7x 106 IU/animal /day for three days prior to slaughter (3D7M); 7x 106 IU/animal /day for six days prior to slaughter (6D7M); 7x 106 IU/animal /day six days followed by seven days of no supplementation prior to slaughter (6D7M7N) and 1x106 IU/animal /day for nine days prior to slaughter (9D1M). Zilpaterol was withdrawn from feed four days prior to slaughter. The animals were humanely slaughtered at the research slaughter facilities of the ARC. The dressed carcasses were split in half and the left side was electrically stimulated for 30 seconds (400 V peak, 5 ms pulses at 15 pulses per second) within 30 minutes post mortem to test the effect of electrical stimulation. The carcasses were chilled at 0 – 5 °C and sampled on the day following slaughter. All samples were collected from the loin (M. longissimus lumborum), vacuum packed and aged for either three or 14 days and were then frozen at -20°C. Frozen loin samples were cut into 30 mm steaks, thawed at 4°C for 24 h and prepared according to an oven-broiling method using direct radiant heat [1]. The steaks were broiled at 260°C (pre-set) to 70°C internal temperature. Six round cores (12.7 mm diameter) were removed from the steaks parallel to the long axis of the muscle fibers and sheared perpendicular to the fiber direction, by a Warner Bratzler shear device mounted on an Instron Universal Testing aparatus [2](cross-head speed set at 200 mm/min). Myofibril fragment lengths (MFL) of loins aged for three and 14 days post-mortem were measured by means of a video image analyses (Soft Imaging System, Olympus, Japan). Myofibrils were extracted according to [3] as modified by [7]. Hundred myofibril fragments per sample were examined and measured with an Olympus BX40 system microscope at a 400X magnification. Data were subjected to analysis of variance for a split-plot design (GenStat, 2003) with the two stimulation treatments as whole plots and the two ageing periods as sub-plots. Means for the interactions between the sub-plot and whole plot were separated using Fishers' protected t-test least significant difference (LSD) at the 5 % level of significance.

III. RESULTS AND DISCUSSION

WBSF was significantly influenced by treatment ($P<0.001$), stimulation ($P<0.001$) and aging ($P<0.001$)(not published) and both aging ($P<0.001$) and stimulation ($P<0.001$) showed significant interactions with treatment . Irrespective of ES or duration of aging, Vitamin D3 did not counteract the negative effect of the zilpaterol on WBSF of the loin. On average, Z increased WBSF by almost 2.5 kg when carcasses were not electrically (NES) stimulated or aged for 3 days (worst scenario). Surprisingly, certain vitamin D3 treatments gave higher ($P<0.05$) WBSF values than Z and other vitamin D3 treatments when loins were not stimulated or were aged for shorter periods (Fig 1 and 2). In both cases 6D7M measured the highest and 9D1M the lowest WBSF. Both extended ageing (14 days) and electrical stimulation (ES) alleviated some of the negative effects of zilpaterol and zilpaterol combined with vitamin D3 and reduced the difference in WBSF between C and the other treatments to ~1.5 kg (Figure 1 and 2). Myofibril fragmentation (MFL) was significantly influenced by treatment ($P<0.001$), ageing ($P<0.001$) and ES ($P<0.001$)(not published). Prolonged aging decreased MFL but ES loins had longer MFL's on average than those of NES loins. A significant interaction between treatment and aging ($P<0.029$; Figure 4) showed that the difference in mean MFL between C compared to Z and vitamin D treatments at three days post mortem generally increased over additional 11 days aging, except for 6D7M7N. The latter was the closest to C for both three and 14 days aging and showed significantly shorter MFL's than 3D7M, 9D1M and Z at 14 days aging. Although the interaction between treatment and stimulation was not significant, figure 3 indicates that 6D7M7N had the shortest MFL of all Z and vitamin D treatments and did not differ from C for ES or NES. Many reports have indicated positive effects on tenderness with high levels of vitamin D3 during the final days before slaughter (e.g. [13], [14], [20]). In most cases the intention was to improve tenderness of meat in general, although [14] and [20] evaluated

the effectiveness of Vitamin D3 to improve meat tenderness of animals with a disposition towards tenderness, such as Bos indicus cattle which would agree with the objective of the present trial. [14] found a general improvement in tenderness irrespective of breed type, (Bos indicus, Bos Taurus), while [20] only focussed on Bos indicus and likewise found positive results. In both studies increased serum and muscle calcium was found which were related to improved proteolyses, although increased calpain activities were found in some but not all studies. In contrast, [12] found no significant effect on tenderness of Brahman cattle when supplementing a vitamin D metabolite and contributed the lack of response to inadequate loading of muscle cells with calcium combined with an elevated calpastatin activity in Brahman loin muscle. Elevated levels of calpastatin activity are thought to play a major role in limiting the action of the calpains and subsequent extent of post mortem proteolysis in Bos indicus breeds of cattle [18]. It is well known that blood calcium is closely regulated by homeostatic mechanisms in the animal body, and it would seem that correct timing and duration of supplementing vitamin D3 could be paramount in elevating muscle calcium to concentrations by which myofibrillar tenderness is improved. In the present trial where elevated calpastatin activity due to beta agonist treatment is also expected [11], it is yet to be verified with further tests whether the various combinations of treatments succeeded in elevating the calcium levels to generate sufficient calpain activity. If MFL can be considered as a good indicator of proteolyses, 6D7M7N and 6D7M treatments tended to be more successful to increase proteolyses with extended aging time than the other treatments (Figure 4). However, considering the high WBSF of these two treatments after 14 days aging, suggest that MFL in the present study do not reflect all the changes in muscle structure leading to final WBSF values, which agrees with [4] who reported a similar lack of correspondence between MFL and tenderness variation. The trend towards higher WBSF values for NES and shorter aged loins of certain vitamin D treatments compared to Z was unexpected. [17] reported higher shear values for 14 and 21 day aged loin muscles of cull cows supplemented 5.0 x106 IU vitamin D3 per animal per day, but gave no explanation for these results. Further tests on calcium level, calcium regulating hormones and proteolytic enzyme activities currently underway should shed some light on this phenomenon for the present study. The fact that both ES and prolonged aging decreased the effect of zilpaterol and reduced the variation among Z and its combinations with vitamin D3 is supported by the study of Strydom, while [5] showed that ES also improved meat tenderness of Bos indicus proportionally more than Bos taurus breeds suggesting that ES is more beneficial in tenderness impaired subjects. Once again this was not supported by MFL in the present trial since ES loins showed longer MFL suggesting less proteolyses and supposedly a lower degree of aging [15].

IV. CONCLUSION

This study showed that various levels and durations of vitamin D3 failed to overcome the tenderness problem often experienced with beta agonists in beef. It is yet to be determined why some vitamin D3 treatments tended to increase WBSF and it is speculated that the timing and duration of supplementation could have caused calcium levels below normal due to over compensation for initial responses to high vitamin D3 levels. Indiscriminate usage of vitamin D3 could result in toxic levels of metabolites and the results of this trial shows that safer methods like electrical stimulation could at least reduce the negative effect of beta agonists although it is not cancelled out completely. Key to graphs:

1. Treatments: C = no beta agonist or vitamin D3; Z = 0.15 mg/kg live weight zilpaterol hydrochloride for thirty days prior to slaughter; 3D7M = Z + vitamin D3 at the 7 x 106 IU/animal /day for three days prior to slaughter; 6D7M = 7 x 106 IU/animal /day for six days prior to slaughter; (6D7M7N) = 7x 106 IU/animal /day six days followed by seven days of no supplementation prior to slaughter; (9D1M) = 1x106 IU/animal /day for nine days prior to slaughter.

2. a,b,c,dBars for six treatments within stimulation or aging sub-groups differ significantly, $P<0.05$)

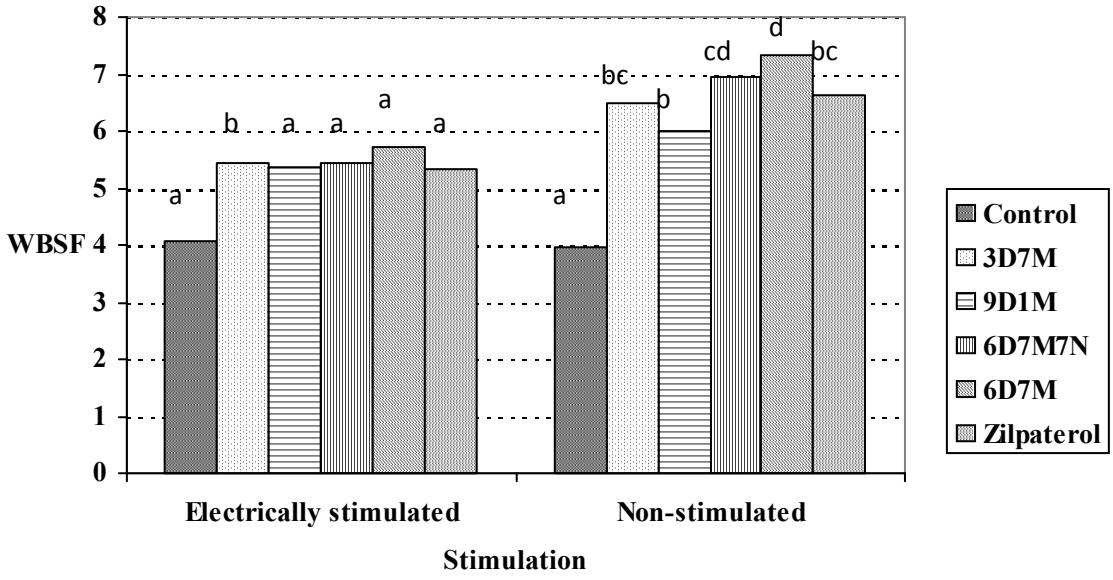

Figure 1:Interaction between treatment and electrical stimulation for Warner Bratzler shear force (WBSF)(SEM = 0.2501)

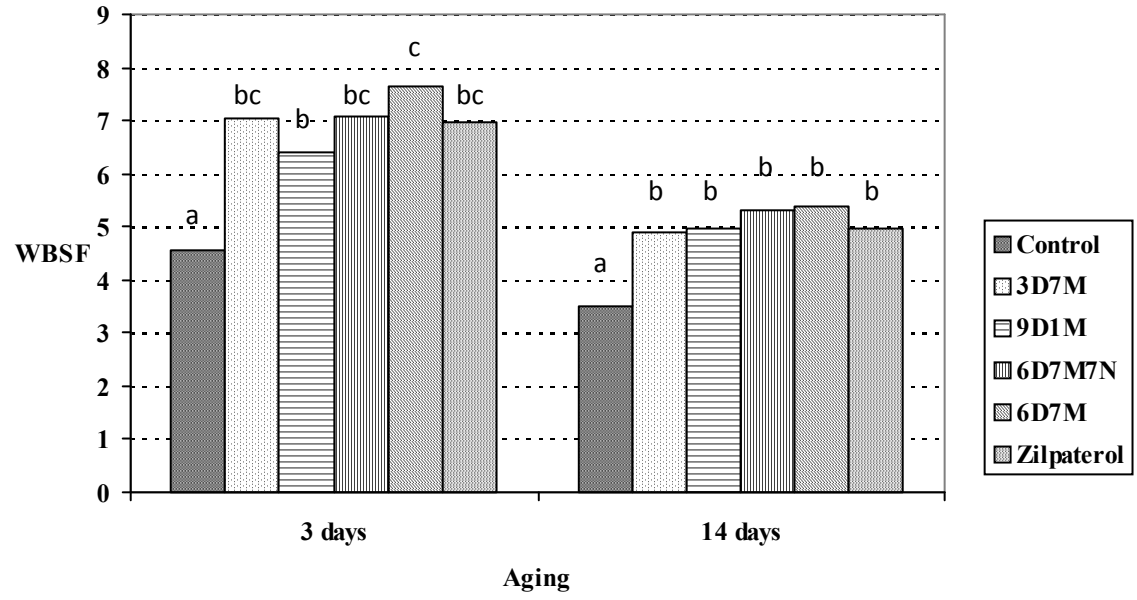

Figure 2: Interaction between treatment and post mortem aging for Warner Bratzler shear force (WBSF)(SEM = 0.1534)

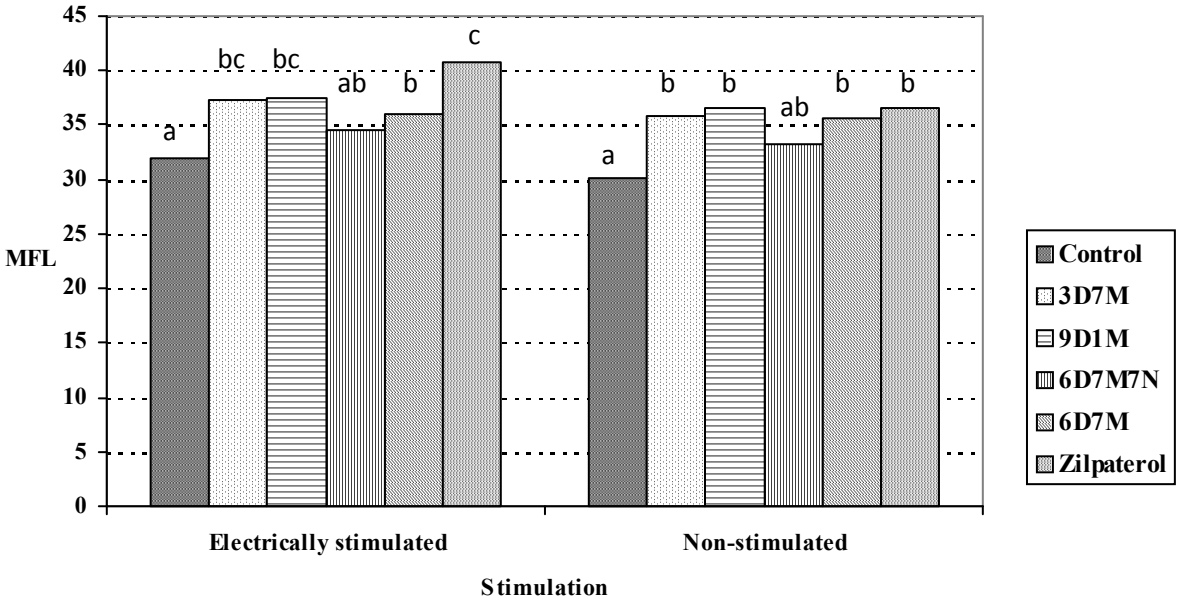

Figure 3: Interaction between treatment and electrical stimulation for myofibril fragment length (MFL)(SEM = 0.683)

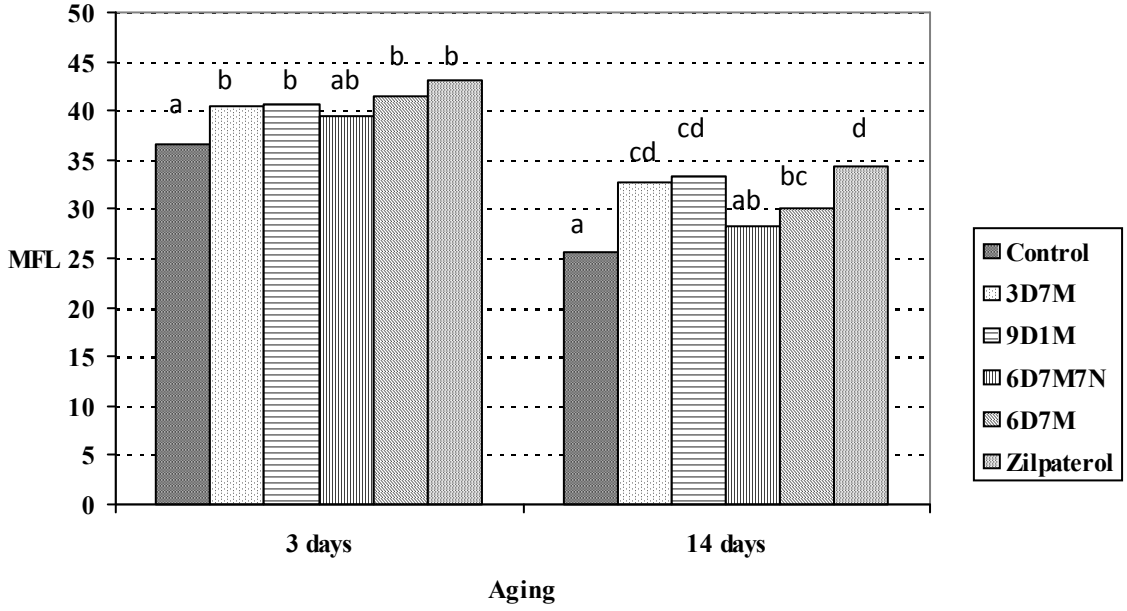

Figure 4: Interaction between treatment and postmortem aging for myofibril fragment length (MFL)(SEM = 0.807)